

**WE CLAIM:**

1. A separation method comprising the steps:
  - (a) detecting in a fluid having a surface, and containing a plurality of localized volumes having a different acoustic impedance than the fluid, a single localized volume located sufficiently near the surface for ejection;
  - (b) determining whether the single localized volume possesses one or more properties;
  - (c) selecting the single localized volume for ejection from the fluid based on the determination of one or more properties in step (b); and
  - (d) ejecting the single localized volume from the fluid by use of focused energy.
2. The method of claim 1, wherein the focused energy is focused acoustic energy.
3. The method of claim 1, wherein the focused energy is focused electromagnetic energy.
4. The method of claim 1, wherein the localized volume comprises a solid or gel particle.
5. The method of claim 1, wherein the localized volume comprises a cell.
6. The method of claim 5, wherein the localized volume comprises a living cell.
7. The method of claim 1, wherein the localized volume is ejected in a trajectory substantially perpendicular to the fluid surface.
8. The method of claim 1, wherein the localized volume is ejected with a non-zero velocity component perpendicular to the fluid surface and a non-zero velocity component parallel

to the fluid surface to effect a trajectory whereby the localized volume experiences a net displacement in a direction parallel to the fluid surface.

9. The method of claim 8, wherein the trajectory is directionally controllable, so that the direction of net displacement parallel to the fluid surface is also directionally controllable.

10. The method of claim 8, wherein the non-vertical distance of travel parallel to the fluid surface is controllable by varying the focused energy.

11. The method of claim 8, wherein the vertical distance of travel parallel to the fluid surface is controllable by varying the focused energy.

12. The method of claim 11, wherein the determining in step (b) of the one or more properties is a quantitative or semiquantitative determination and the selecting of step (c) is between non-ejection and multiple ejection trajectories and depends upon the quantitative or semiquantitative determination.

13. The method of claim 12, wherein the fluid is contained in a fluidic channel.

14. The method of claim 13 wherein data from said detecting of (a) and said determining of (b) is input into a processor, whereby the processor directs said selecting of (c) and said ejecting of (d) by reference to the measured data, programmed selection criteria, and system parameters.

15. The method of claim 1 wherein the localized volumes are circumscribed volumes comprising living cells.

16. A system for the separation, from a carrier fluid having a fluid surface and containing a plurality of circumscribed volumes having a different acoustic impedance than the carrier fluid, of one or more of the circumscribed volumes, the system comprising:

a fluidic container;  
a detector for detecting the localized volume and determining a property of the localized volume; and

an acoustic ejector of fluid droplets from the carrier fluid, comprising an acoustic  
5 radiation generator for generating acoustic radiation and a focusing means for focusing the acoustic radiation at a focal point near the fluid surface;

wherein the circumscribed volumes present in the carrier fluid that are detected to be sufficiently near the fluid surface for ejection may be acoustically ejected to a target from the carrier fluid in a fluid droplet depending upon whether the circumscribed volume possesses one or  
10 more properties.

17. The system of claim 16 further comprising a means for positioning the ejector in acoustic coupling relationship to the container in an appropriate position to permit the focusing means to focus the acoustic radiation at the focal point in a desired point of the carrier fluid.

18. The system of claim 17 further comprising a processor for integrating said detector, said acoustic ejector, and said means for positioning said acoustic ejector with respect to said container to eject the circumscribed volumes to selected targets based upon the detected property.

19. The system of claim 18 wherein said fluidic container comprises a fluidic channel that has a surface, the fluidic channel having dimensions permitting the carrier fluid containing the plurality of circumscribed volumes to flow freely through the channel.

20. The system of claim 19 wherein said fluidic channel has dimensions permitting the  
25 plurality of circumscribed volumes, or a subset of the plurality of circumscribed volumes, to flow freely through the channel only in substantially single file.

21. The system of claim 19 wherein the target is an array site or a target fluidic channel.

22. The system of claim 21 wherein based upon a quantitative or semiquantitative measurement of the property by said detector the circumscribed volume is selected not to be ejected, to be ejected into a target channel or to be ejected onto an array site.

5 23. The system of claim 22 wherein the array site is a well plate well.

24. The system of claim 22 wherein the circumscribed volume ejected into the target channel may be ejected from the target channel into a subsequent target channel or a subsequent array site.

10 25. The system of claim 16 or 22 wherein said circumscribed volume is a cell.

26. The system of claim 25 wherein the cell is a living cell.

15 27. A system for the separation, from a carrier fluid having a surface containing a plurality of circumscribed volumes having a different acoustic impedance than the carrier fluid, of one or more of the circumscribed volumes, the system comprising:

a container;

a substrate having a substrate surface oriented substantially parallel to the surface;

20 means for acoustically ejecting from the carrier fluid through the aperture onto a location on the substrate surface a circumscribed volume having a different acoustic impedance than the carrier fluid,

wherein the circumscribed volume present in the carrier fluid that is detected near the fluid surface below the aperture may be acoustically ejected from the carrier fluid onto a substrate location in a fluid droplet depending upon whether the localized volume possesses one or more properties.

25 28. The system of claim 27 further comprising a processor for integrating said detector, said acoustic ejection means, and said means for positioning said acoustic ejector with respect to

said container to eject the circumscribed volumes to selected targets based upon the detected one or more properties.

29. The system of claim 28 wherein said fluidic container comprises a fluidic channel  
5 that has a surface, the fluidic channel having dimensions permitting the carrier fluid containing the plurality of circumscribed volumes to flow freely through the channel.

30. The system of claim 29 wherein said fluidic channel has dimensions permitting the  
10 plurality of circumscribed volumes, or a subset of the plurality of circumscribed volumes, to flow freely through the channel only in substantially single file.

31. The system of claim 29 wherein the target is an array site on said substrate surface  
or a target fluidic channel.

32. The system of claim 31 wherein based upon a quantitative or semiquantitative  
15 measurement of the property by said detector the circumscribed volume is selected not to be ejected, to be ejected into a target channel, or to be ejected onto an array site.

33. The system of claim 32 wherein the array site is a well plate well.  
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34. The system of claim 32 wherein the circumscribed volume ejected into the target  
channel may be ejected from the target channel into a subsequent target channel or a subsequent  
array site.

35. The system of claim 27 or 32 wherein said circumscribed volume is a cell.  
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36. The system of claim 35 wherein the cell is a living cell.

37. A method for ejecting one or more cells from a colony of cells disposed on a medium surface to a target comprising a substrate surface or a container, the method comprising locating a colony and ejecting the cells by focused energy.

5 38. The method of claim 37 wherein ejection is by focused acoustic energy.

39. The method of claim 38 wherein the locating is effected by measuring acoustic impedance at the medium surface.

10 40. The method of claim 37 or 39 wherein the medium is a gel or semisolid, further comprising causing the medium to liquefy in a volume transected by a plane parallel to the medium surface and wholly underlying the boundaries of the colony of cells.

15 41. A system for ejecting cells from a colony of cells disposed on a surface of a medium, comprising:  
an acoustic energy source;  
means for focusing acoustic energy from said acoustic energy source to a focal point;  
means for positioning the focal point at any point in the medium or the colony of cells,  
wherein the cells are ejected by delivering acoustic energy to the focal point with an  
20 adequate power and for a sufficient time to deliver a quantity of acoustic energy that ejects the cells.

42. The system of claim 41 further comprising a processor for integrating said acoustic energy source, said means for focusing acoustic energy, and said means for positioning the focal  
25 point at any point in the medium to eject the cells to selected targets from the cell colonies.

43. The system of claim 41 wherein the colony of cells is located by scanning the focal point across the surface to detect an area of the surface having a different acoustic impedance than an area of the surface without any colony of cells.

44. The system of claim 41 or 42 wherein the medium comprises a gel and acoustic energy is additionally delivered to the bulk of the medium prior to cell ejection into a volume transected by a plane parallel to the medium surface and wholly underlying the boundaries of the colony of cells.

45. The system of claim 44 wherein the acoustic energy is delivered to the volume at the geometric center of the volume, the geometric center located a distance beneath the surface of the medium in the bulk.

46. The system of claim 44 wherein the geometric center of the volume is located about 50 to 150  $\mu\text{m}$  beneath the surface of the medium in the bulk.

47. The system of claim 41 wherein the medium in the volume undergoes a change prior to ejection from the geometric center of the volume to the surface of the medium, whereby the change reduces the quantity of acoustic energy to eject the cells.

48. The system of claim 47 wherein the change is a liquefying and the liquefying is detected by measuring a change in acoustic impedance or acoustic attenuation of the volume.

49. The system of claim 42 wherein the target is an array site or a fluidic channel.

50. The system of claim 48 wherein the liquefying is caused by deposition of a reagent, a characteristic of the cells in the colony, or the delivery of energy to the medium.

51. The system of claim 42 further comprising a detector that measures a property of the colony of cells.

52. The system of claim 51 wherein based upon a quantitative or semiquantitative measurement of the property by said detector the cells from the colony are selected not to be ejected, to be ejected into a target container, or to be ejected onto an array site.

5 53. The system of claim 52 wherein the array site is a well plate well.

54. The system of claim 52 wherein the target container is a fluidic channel.

10 55. The system of claim 52 or 54 wherein cells ejected into the target container may be ejected from the target container into a subsequent target container or a subsequent array site.

56. The system of claims 41, 42, or 52 wherein the cells are living cells.

15 57. A method for conditioning a material to facilitate subsequent acoustic ejection in a delineated volume of material comprising delivering acoustic energy to a focal point in the material.

58. The method of claim 57 wherein the material at the focal point is heated.

20 59. The method of claim 57 wherein the material at the focal point undergoes a phase transition.

60. The method of claim 57 wherein the material is a gel, amorphous solid, or semisolid and the phase transition at the focal point is to a liquid.

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61. The method of claim 60 wherein the material is an agar gel having a surface and the focal point is below the surface of the agar gel and beneath a colony of cells.



62. A method for delivering thermal energy to a delineated volume of material comprising delivering acoustic energy to a focal point in the material.

63. The method of claim 62 wherein the material at the focal point is heated.

64. The method of claim 62 wherein the material at the focal point undergoes a phase transition.

65. The method of claim 62 wherein the material is a gel, amorphous solid, or semisolid and the phase transition at the focal point is to a liquid.

66. The method of claim 65 wherein the material is an agar gel having a surface and the focal point is below the surface of the agar gel and beneath a colony of cells.